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ORIGINAL PAPER

X1-homologous genes family as central components in biotic and abiotic stresses response in maize (*Zea mays* L.)

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Abstract *X1*-homologous genes (XHS) encode plant specific proteins containing three basic domains (XH, XS, zf-XS). In spite of their physiological importance, systematic analyses of *ZmXHS* genes have not yet been explored. In this study, we isolated and characterized ten *ZmXHS* genes in a whole-of-genome analysis of the maize genome. A total of ten members of this family were identified in maize genome. The ten *ZmXHS* genes were distributed on seven maize chromosomes. Multiple alignment and motif display results revealed that most ZmXHS proteins share all the three conserved domains. Putative *cis*-elements involved in abiotic stress responsive, phytohormone, pollen-specific and quantitative, seed development and germination, light and circadian rhythms regulation, Ca^{2+}-responsive, root hair cell-specific, and CO_2-responsive transcriptional activation were observed in the promoters of *ZmXHS* genes. Yeast hybrid assay revealed that the XH domain of ZmXHS5 was necessary for interaction with itself and ZmXHS2. Microarray data showed that the *ZmXHS* genes had tissue-specific expression patterns in the maize developmental steps and biotic stresses response. Quantitative real-time PCR analysis results indicated that, except *ZmXHS*9, the other nine *ZmXHS* genes were induced in the seedling leaves by at least one of the four abiotic stresses applied.

Keywords Maize · Abiotic and biotic stress · *X1*-homologous genes · Expression behavior · *cis*-elements

Abbreviations

ABA	Abscisic acid
bZIP	Basic leucine zipper
DAP	Day after pollination
ORF	Open reading frame
PEG	Polyethylene glycol
RDR6	RNA-dependent RNA polymeraes 6
XH	X1 homologue
XHS	XH and XS domain

Introduction

X1-homologous genes (*XHS*) encode plant specific proteins containing three basic domains XH, XS, and zf-XS which were named after *Arabidopsis* SGS3 (Suppressor of Gene Silencing 3) and rice homolog X1 (Mourrain et al. 2000; Bateman 2002; Qin et al. 2009). Among these protein domains, zf-XS motif is a C2H2 type zinc finger domain, the XS motif is an RNA-binding domain and XH motif refers to X-homolog domain with unknown function (Bateman 2002). Furthermore, these protein domains, some of SGS3-like proteins also include a coil–coil domain localized between the XS and XH domains (Ausin et al. 2009; Zheng et al. 2010).

The main region of rice X1 and *Arabidopsis* SGS3, XS domain, is around 140 amino acid residues in length. The XS domain contains an absolutely conserved aspartate that could present an enzymatic active site (Bateman 2002). The XS domain containing proteins are predicted by ncoils to contain coiled-coils (Lupas et al. 1991), which indicate that they could

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oligomerise. Like most coiled-coil proteins (which could form a dimeric or a trimeric structure), different members of the XS domain family may oligomerise via their coiled-coils to form a variety of complexes (Bateman 2002). The XH domain is between 124 and 145 residues in length. The XH domain contains one absolutely conserved glutamate which could potentially be an active site or other functionally vital region. XS and XH domains are found in most of these proteins and two domains may interact (Bateman 2002; Xie et al. 2012a). The zf-XS domain is an N-terminal cysteine/histidine zinc binding domain and usually accompanies a XS domain (Bateman 2002).

Up to now, some experimental data have been reported indicating the functions of this family, such as SGS3 in *Arabidopsis*, OXHS in rice and SISGS3 in tomato (Mourrain et al. 2000; Glick et al. 2008; Qin et al. 2009). SGS3 is essential for post-transcriptional gene silencing and natural virus resistance (Mourrain et al. 2000; Xie et al. 2012b), which are vital for endogenous gene silencing from DNA viruses (Muangsan et al. 2004; Qin et al. 2009) and the production of trans-acting siRNAs in *Arabidopsis* (Peragine et al. 2004). SGS3 acts upstream of RDR6-dependent dsRNA production (Yoshikawa et al. 2005) and the XS domain probably acts as an RNA recognition motif (Bateman 2002; Zhang and Trudeau 2008; Elmayan et al. 2009). Along with the function of SGS3 in *Arabidopsis* antiviral response, SISGS3 is targeted by the tomato yellow leaf curl geminivirus PTGS suppressor protein V2 (Glick et al. 2008). Nine rice OXHS genes are responsive to at least one of the abiotic stresses including salt, drought, cold, and abscisic acid treatment (Qin et al. 2009).

These reports in plants suggested that *XHS* family genes may be crucial not only for plant development but also for response and adaptation to stresses. So, in order to indicate the functions of Zm*XHS* gens in this family, transcript expression levels of the family genes were investigated in various maize tissues and in the seedling leaves under various abiotic and biotic stresses. The results presented in this study gave an important reference for function studies of *XHS* family genes in maize.

Materials and methods

Isolation of *XHS* gene in maize

The sequences of 14 *Arabidopsis* and 11 rice XHS proteins were downloaded from the TAIR database (http://www.arabidopsis.org) and TIGR database (http://rice.plantbiology.msu.edu) (Qin et al. 2009). To obtain all the *XHS* genes in maize, BLASTP searches were performed in the Maize sequence database (http://www.maizesequence.org/index.html) and Gramene database (http://www.gramene.org/Multi/blastview) with the *Arabidopsis* and rice XHS proteins as queries. First, all the corresponding protein sequences of the putative XHS family members were downloaded if it satisfied with $E<10^{-10}$ and including XH domain (accession no. PF03469), XS domain (accession no. PF03468), or zf-XS domain (accession no. PF03470). And then, all the candidate proteins were confirmed with the Pfam database (http://www.sanger.ac.uk/Software/Pfam/search.shtml) (Sonnhammer et al. 1997). The full-length cDNA sequences of *ZmXHS* genes in maize were downloaded from the Maize sequencing database.

Gene structure analysis of maize *XHS* genes

The information of all *ZmXHS* genes, including chromosomal location, full-length DNA sequences were obtained from the B73 maize sequencing database (http://www.maizesequence.org/index.html). Exons and introns structures of *ZmXHS* genes were confirmed by using GSDS (http://gsds.cbi.pku.edu.cn/) (Guo et al. 2007).

Motif display analysis of ZmXHS proteins

Multiple Expectation Maximization for Motif Elicitation (MEME) utility program (Bailey et al. 2009) was used to display domains of ZmXHS, OsXHS, AtXHS, and SbXHS proteins. The matrix for phylogenetic analysis included the 14, 11, 19, and 10 *XHS* genes from *Arabidopsis*, rice, sorghum and maize.

Promoter regions analysis of *ZmXHS* genes

To investigate *cis*-elements in promoter sequences of *ZmXHS* genes, 2 kb of B73 genomic DNA sequences upstream of the initiation codon (ATG) were downloaded from NCBI. Promoter structure was predicted using Promoter 2.0 software (http://www.cbs.dtu.dk/services/Promoter/) and PLACE (Plant *cis*-acting Regulatory DNA Elements, with more than 6 bp) (http://www.dna.affrc.go.jp/PLACE/) (Higo et al. 1999).

Yeast two-hybrid assays

A Gal4-based two-hybrid system was used as described by the manufacturer (Clontech). The full-length and truncated ZmXHS5 (ZmXHS5-1, lacking the XH domain; ZmXHS5-2, XH domain alone) coding region were amplified by PCR with primers containing restriction sites and cloned into the pGADT7 cloning vector (Clontech) to make the bait plasmid pGADT7-ZmXHS. The coding sequences of the ZmXHS2 and ZmXHS5 were cloned into the DNA binding domain vector pGBKT7. The primers and restriction sites used to create these constructs are listed in Supplementary Table 1. Each pGBKT7-ZmXHS was separately co-transformed with pGADT7-ZmXHS5/ZmXHS5-1/ZmXHS5-2 into

Saccharomyces cervisiae AH109 by the LiAc-mediated yeast transformation method according to the Yeast Protocols Handbook of Clontech. Co-transformed yeast cells were selected on synthetic medium lacking Leu and Trp and interaction was selected on synthetic medium lacking Leu, Trp, His, and Ade.

Plant materials stress treatment

Seeds of the maize (*Zea mays*) B73, were surface-sterilized, germinated and hydroponically grown to the three-leaf stage in pots filled with vermiculite, with four seeds per pot. The pots were placed in a greenhouse at 28 °C under 16 h light and 20 °C under 8 h dark and watered once every 3 days. For ABA treatment, leaves were sprayed with 100 μM (+)-*cis*, trans-ABA and then rapped up with preservative film, followed by sampling at 0, 1, 3, 6, 12, and 24 h. Abiotic treatments concluded cold, NaCl and PEG. For cold stress, the seedlings were put into a growth chamber at 4 °C and sampled at 0, 1, 3, 6, 12, and 24 h, respectively. For NaCl and PEG treatment, the seedlings were carefully removed from the vermiculite and washed clean with tap water and then dried using filter paper. Roots of the seedlings were then submerged in 200 mM NaCl or 20 % PEG (molecular weight 6,000) solution, air conditioned by a pump and sampled at 0, 1, 3, 6, 12, and 24 h, respectively (Zhang et al. 2012).

Microarray data collection and analyses of expression profiles

The expression behaviors of *ZmXHS* genes were examined in a set of maize transcriptome data at PLEXdb (http://www.plexdb.org). The microarray data of genome-wide gene expression datas of maize inbred line B73 (GSE27004) and the data of transcriptomic analysis of induced senescence in maize were provided by Kaeppler from University of Wisconsin (Sekhon et al. 2011, 2012). Gene expression data from the Affymetrix GeneChip array data during three kinds of fungal infection were downloaded from GEO with accession numbers, GSE31188, GSE19501, and GSE29747, respectively (Ghareeb et al. 2011; Voll et al. 2011). The data were analyzed by GeneSpring 12.5 software. Heat map was used to present the number of *ZmXHS* genes, and the map was generated using MultiExperiment Viewer (MeV, version 4.8.1) software. The data were adjusted by median centering of genes. The data were clustered by complete linkage clustering method, and a euclidean distance metric was used.

Transcript level analysis *ZmXHS* genes

Total RNA was prepared using the TRIZOL reagent (Invitrogen, Karlsruhe, Germany) and then purified using the DNase I (RNase free) (TaKaRa, Dalian) according to the manufacturer's protocol. After an initial photometric determination of total RNA concentration, ethidium bromide fluorescence of ribosomal bands on the electrophoresis-gel was used for the control of total RNA integrity and the adjustment of equal RNA amounts. Real-time PCR was performed in an optical 96-well plate with an ABI StepOnePlus Real-Time PCR System. Gene-specific primers were designed for all ten *ZmXHS* genes (Table 2) and *ZmGAPDH* transcript served as the control (Kozak 1999). Each reaction contained 10 μl of SYBR Premix Ex Taq (TaKaRa, Dalian), 1.0 μl of cDNA samples, 0.4 μl ROX Reference Dye II and 10 μM gene-specific primers in a final volume of 20 μl. The thermal cycle used was as follows: 95 °C of 30 S, 40 cycles of 95 °C for 5 s and 60 °C for 60 s. For melt curve, 95 °C 15 S, 60 °C for 60 s and 95 °C 15 S. The analysis of real-time PCR data used $2^{-\Delta\Delta C}{}_{T}$ method (Livak and Schmittgen 2001).

Results

Identification of *XHS* family genes in maize

After carefully surveying the maize genome, ten members were defined as *ZmXHS* genes (Table 1). There was no *ZmXHS* gene homologous to *OXHS3* and *OXHS10* but two

Table 1 List of *X1*-homologous genes in maize

Gene name	Chr[a]	ORF length	Protein length	Protein ID
ZmXHS1	3	1,887	628	GRMZM2G160032_P01
ZmXHS2	3	1,593	530	GRMZM2G011436_P01
ZmXHS4	10	1,881	626	GRMZM2G096367_P01
ZmXHS5	1	2,064	687	GRMZM2G110304_P01
ZmXHS6	8	2,076	691	GRMZM2G025059_P01
ZmXHS7	3	2,058	685	GRMZM2G100898_P01
ZmXHS8a	9	1,380	459	GRMZM2G175463_P02
ZmXHS8b	1	762	253	GRMZM2G397339_P01
ZmXHS9	4	1,533	510	GRMZM2G032110_P01
ZmXHS11	6	1,785	594	GRMZM2G020187_P01

[a] Chromosome number in which the gene resides

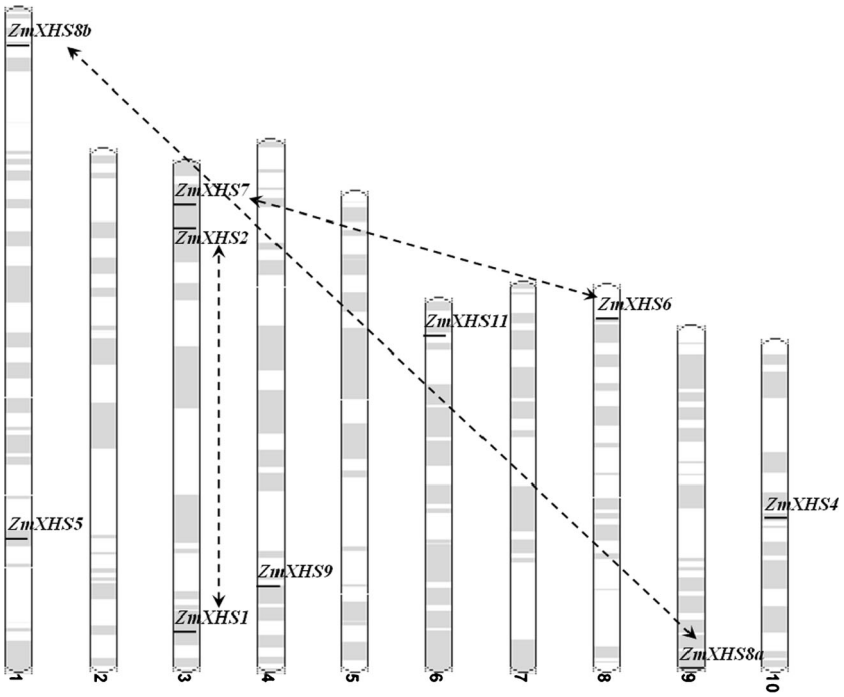

Fig. 1 Chromosomal localization of maize *XHS* genes. Segmental duplicates, including *ZmXHS1*/*ZmXHS2*, *ZmXHS6*/*ZmXHS7*, and *ZmXHS8a*/*ZmXHS8b*

OXHS8 homologous genes named *ZmXHS8a* and *ZmXHS8b*. The length of ZmXHS proteins ranged from 253 aa (amino acids) to 691 aa. BLAST analysis against the Pfam database showed that all of them including XH domain (accession no. PF03469), XS domain (accession no. PF03468), or zf-XS domain (accession no. PF03470) (Table 1).

The ten *ZmXHS* genes were distributed on seven maize chromosomes: chromosome 1 contained two genes; chromosome 3 contained three genes, and chromosome 4, 6, 8, 9, 10 contained one gene, respectively (Fig. 1). Based on phylogenetic results (Supplementary Fig. 1), three paralogs were identified in *ZmXHS* genes including *ZmXHS1*/*ZmXHS2*, *ZmXHS6*/*ZmXHS7*, and *ZmXHS8a*/*ZmXHS8b* (Fig. 1). The full-length cDNA sequences were compared with the corresponding genomic DNA sequences to determine the numbers and positions of exons and introns within each *ZmXHS* gene by using GSDS (http://gsds.cbi.pku.edu.cn/chinese.php) (Guo et al. 2007). The genome structure of the *ZmXHS genes* showed that most *ZmXHSs* had six exons, except for *ZmXHS8a, 8b, 9* which had three (Fig. 2; Table 2).

Motif analysis and protein architecture

To identify the conserved domains distribution in XHS proteins, the MEME web server was employed to analyze the protein sequences from *Arabidopsis*, rice, sorghum and maize. Three putative conserved domains were detected in the

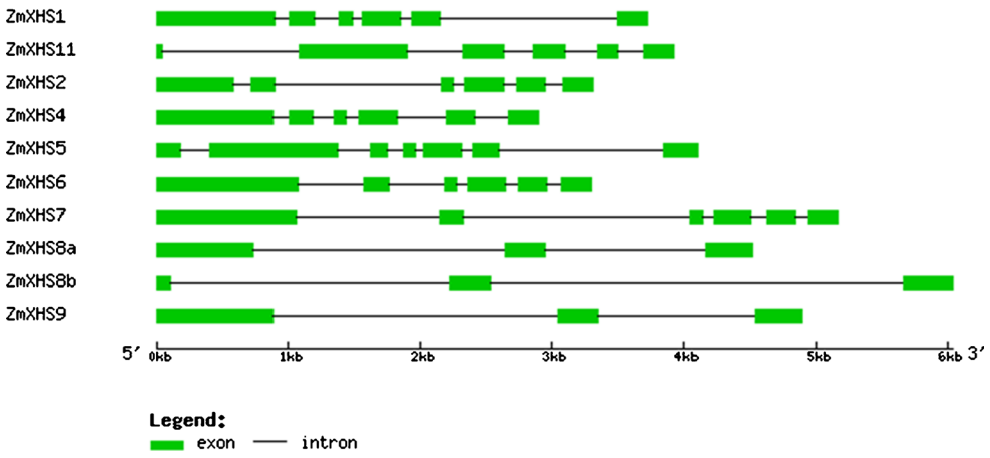

Fig. 2 Gene structure of maize *XHS* gene family. Exons and introns were showed by *filled boxes* and *single lines*, respectively

Table 2 Primers of maize *X1*-homologous genes for real-time PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Tm (°C)
ZmXHS1	TCCAGGAATAATAGCGGACCAC	TCCCTTTGCCCCATCATCTTCT	60
ZmXHS2	CAGTGGGTCAGAGATGTTTGGA	TGTCAAGTGATGCGGTCGTCTC	60
ZmXHS4	AGGTTCTTGTCAGCGAATGGTG	GCAGCAAATCGCTCTCTTCCAT	60
ZmXHS5	AGGCTAACAGCACAGTTCTCGC	AAGCCATCCATAAAGGTCGTCC	60
ZmXHS6	AGGTGAGGTCAAGGTTTATGGG	CAAGTGTCGGTTCTTTTCCTCG	60
ZmXHS7	GATGTCAAGGTTTATGGGTGGG	TTGAGATGGAGAGTTCTGTCGC	60
ZmXHS8a	GCACCGTCCGAACCTACTT	CGAAGCCCTCGTCCTGTTC	60
ZmXHS8b[a]	CATAAAGCCCACCTCCATCAGT	TATTTTGTCCACAGTAGCAGC	60
ZmXHS9	GAAAGTGGAGAAGCAGGTGAAGG	GCACGATGCTCCTGATTCTTT	60
ZmXHS11	AACTTCAGTGTTACCAGCGGG	CTCATACCACGGTGCCCAGAGG	60
ZmGAPDH	CCCTTCATCACCACGGACTAC	AACCTTCTTGGCACCACCCT	60

[a] The primers for *ZmXHS8b* were designed from 3'-UTR (untranslated regions)

ZmXHS family, including XH (PF03469), XS (PF03468), and zf-XS (PF03470) which have been identified in the X1 and SGS3 proteins. According to the configuration of the putative domains, the 10 ZmXHS proteins can be divided into four types (Fig. 3). The protein sequences in type I, including ZmXHS1, ZmXHS2, ZmXHS4, ZmXHS5 and ZmXHS6 contained the XH domain, XS domain, and zf-XS domain. Three proteins ZmXHS8a, ZmXHS8b, ZmXHS9 each containing only the zf-XS domain were classified as type II. Type III protein ZmXHS7 contained XH domain and XS domain, while Type IV protein ZmXHS11 contained XS domain and zf-XS domain.

All these three putative conserved domains were present in most of the XHS members of rice, *Arabidopsis* and sorghum. According to the configuration of the putative domains, the XHS proteins of rice, *Arabidopsis* and sorghum can be divided into three or four types, respectively (Supplementary Fig. 2) (Qin et al. 2009).

The XH domain of ZmXHS5 is necessary for interacts with itself and ZmXHS2

Our previous study indicated that ZmXHS5 interacts with itself and ZmXHS2 (Supplementary Fig. 3). To identify the protein domains of ZmXHS5 responsible for the interaction, we generated two truncation mutants of ZmXHS5 in pGADT7 (Fig. 4a): lacking the XH domain (ZmXHS5-1), the XH domain alone (ZmXHS5-2). We checked the interaction of these truncated ZmXHS5 mutants with full-length ZmXHS5 and ZmXHS2 using the yeast two-hybrid assay described above. ZmXHS5-2 was able to interact with ZmXHS5 and ZmXHS2, because co-transformation of the two pairs enabled yeast cell to grow in the absence of Ade and His (Fig. 4b). However, ZmXHS5-1 did not interact with ZmXHS5 and ZmXHS2. These results indicated that the XH domain of ZmXHS5 is necessary for ZmXHS5–ZmXHS5 and ZmXHS5–ZmXHS2 interactions.

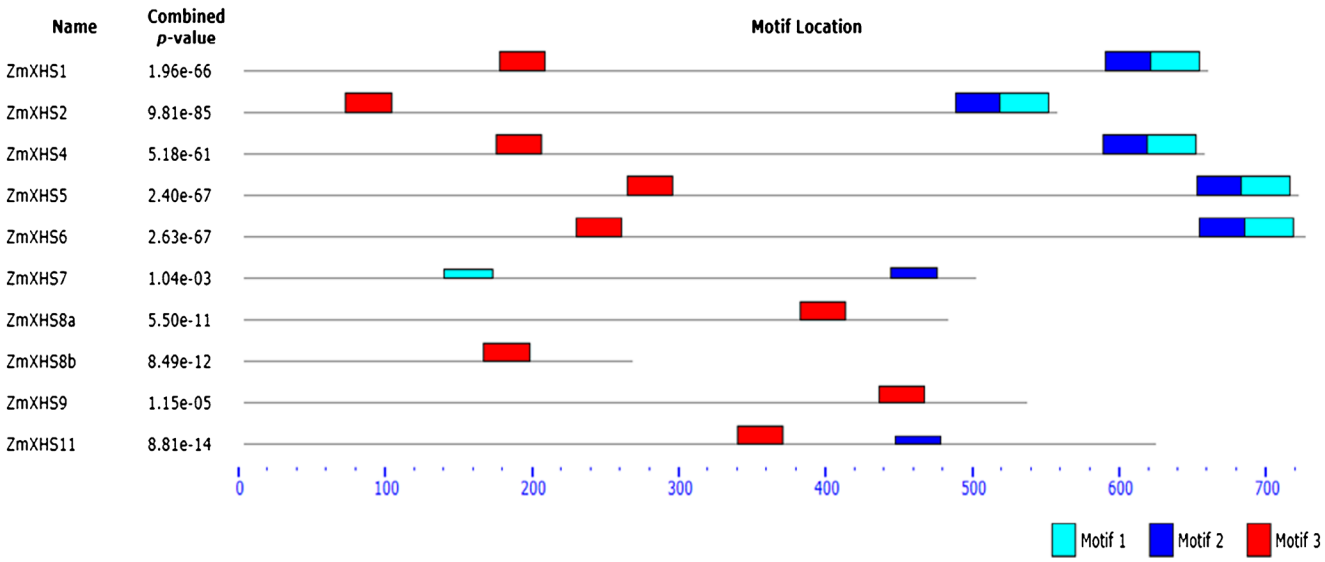

Fig. 3 Putative motif distribution in maize *XHS* proteins. Domains of XHS proteins were investigated by MEME web server. *Motif1* XH; *Motif2* XS; *Motif3* zf-XS

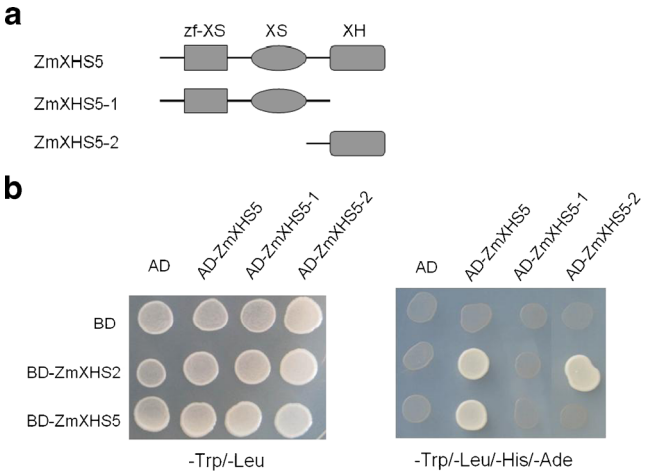

Fig. 4 The XH domain mediates ZmXHS5–ZmXHS5 and ZmXHS5–ZmXHS2 interactions **a** Schematic structure of the full-length and truncated ZmXHS5 proteins used for yeast two-hybrid assays. ZmXHS5-1, truncated ZmXHS5 protein lacking the XH domain; ZmXHS5-2, XH domain alone; **b** interaction analyses of truncated ZmXHS5 proteins with ZmXHS5 and ZmXHS2 in yeast AH109 cells. The paired AD and BD fusion constructs were co-transformed into yeast. Positive clones selected on –Leu/–Trp were spotted on –Ade/–Leu/–Trp/-Ade medium

cis-Element analysis

By searching the PLACE database, promoter regions (2kp range B73 genomic DNA sequences upstream of translation start site) of *ZmXHS* genes were analyzed. Amounts up to 58 putative *cis*-elements more than 6 bp length were identified (Supplementary Table 2). In this study, a series of *cis*-elements were found that involved in abiotic stress responsive, phytohormone, pollen-specific and quantitative, seed development and germination, light and circadian rhythms regulation, Ca^{2+}-responsive, root hair cell-specific, and CO_2-responsive transcriptional activation.

Among these *cis*-elements, five kinds were distributed in all the ten *ZmXHS* genes, i.e., the dehydration-stress responsive element MYBCORE and MYCCONSENSUSAT (Solano et al. 1995; Abe et al. 2003), Ca^{2+}-responsive element CGCGBOXAT (Yang and Poovaiah 2002) and EECCRCAH1 (Guo et al. 2010), ABA-responsive element DPBFCOREDCDC3 (Kim et al. 1997; Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000), light-responsive element EBOXBNNAPA and INRNTPSADB (Stalberg et al. 1996; Hartmann et al. 2005), SA-responsive element GT1CONSENSUS (Villain et al. 1996; Le Gourrierec et al. 1999). Another widely distributed *cis*-element ABRERATCAL (Kaplan et al. 2006), CO_2-responsive element was present in nine of the ten *ZmXHS* genes. Pollen-specific and quantitative element QELEMENTZMZM13 (Hamilton et al. 1998), phytochrome regulation element REBETALGLHCB21 (Degenhardt and Tobin 1996), root hair cell-specific element RHERPATEXPA7 (Kim et al. 2006), early responsive to dehydration element MYCATERD1 (Tran et al. 2004), fermentative pathway responsive element ANAERO1CONSENSUS (Bratić et al. 2009), also exist in most of the *ZmXHS* gene promoters.

By controlling efficiency of the promoters, *cis*-elements played essential function in the regulation of gene expression. Studies on *cis*-elements could provide vital foundation for further functional research of the *ZmXHS* gene family.

Expression profiles of *ZmXHS* family in different tissues and organs

In order to identify the spatial and temporal specific expression patterns of *ZmXHS* genes, we explored microarray data which record the gene expression levels of 60 tissues

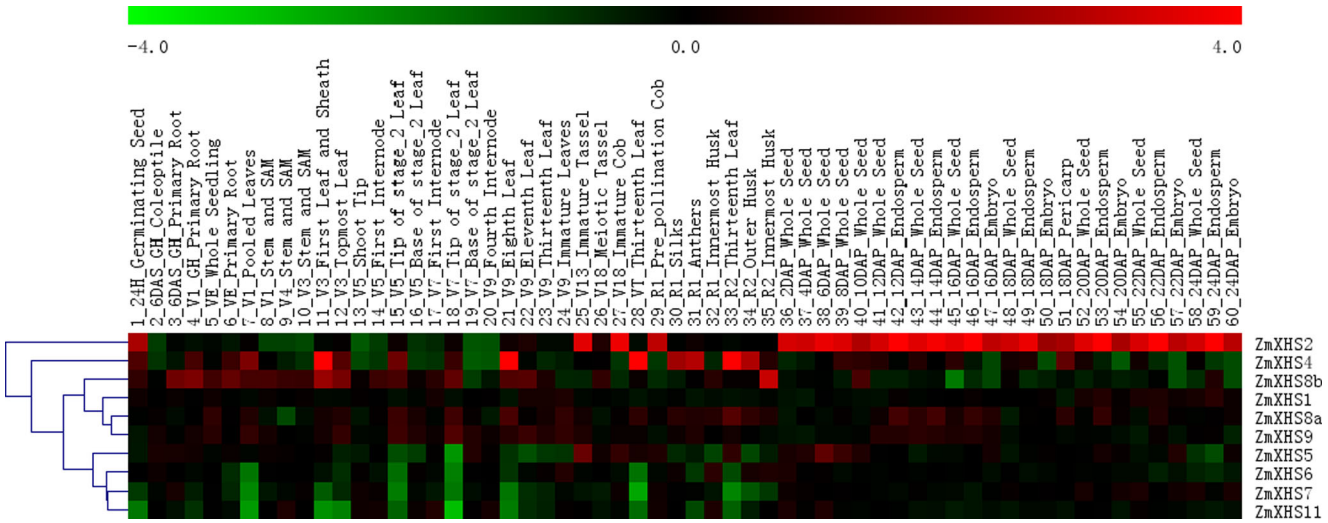

Fig. 5 Organ-specific expression patterns of *ZmXHS* genes detected in the microarray data. Log2 ratios of expression were used to make this heat map. *Red color* indicates higher expression while *green color* signifies lower expression in 60 different tissues

varying developmental stages of the maize (Sekhon et al. 2011). It can be seen from the heat map that all of the ten detected genes were involved in numerous biological processes, and expressed in almost tissues, but their expression levels were distinct (Fig. 5). *ZmXHS2* had higher expression in reproductive organs such as cob, tassel, whole seed (DAP), endosperm (DAP), and embryo (DAP). The transcript level of *ZmXHS4* was detected at the higher level in silks, anthers, sheath, outer husk, and different leaves. *ZmXHS8a* and *ZmXHS9* had similar expression pattern but different from that of *ZmXHS8b*. *ZmXHS8b* was involved in the development of vegetative organs such as primary root, stem, SAM, sheath, internode, and innermost husk, while *ZmXHS8a* and *ZmXHS9* had higher expression both in reproductive and vegetative organs, i.e., seed, endosperm, embryo, leaf, stem, and husk. *ZmXHS5-7* and *ZmXHS11* had similar expression pattern, appearing to be lowly expressed among vegetative organs and moderate expressed among reproductive organs (Fig. 5).

Expression profiles of *ZmXHS* genes under abiotic stresses

Many plant gene families were involved both in stress and development responses. In order to check whether *ZmXHS*

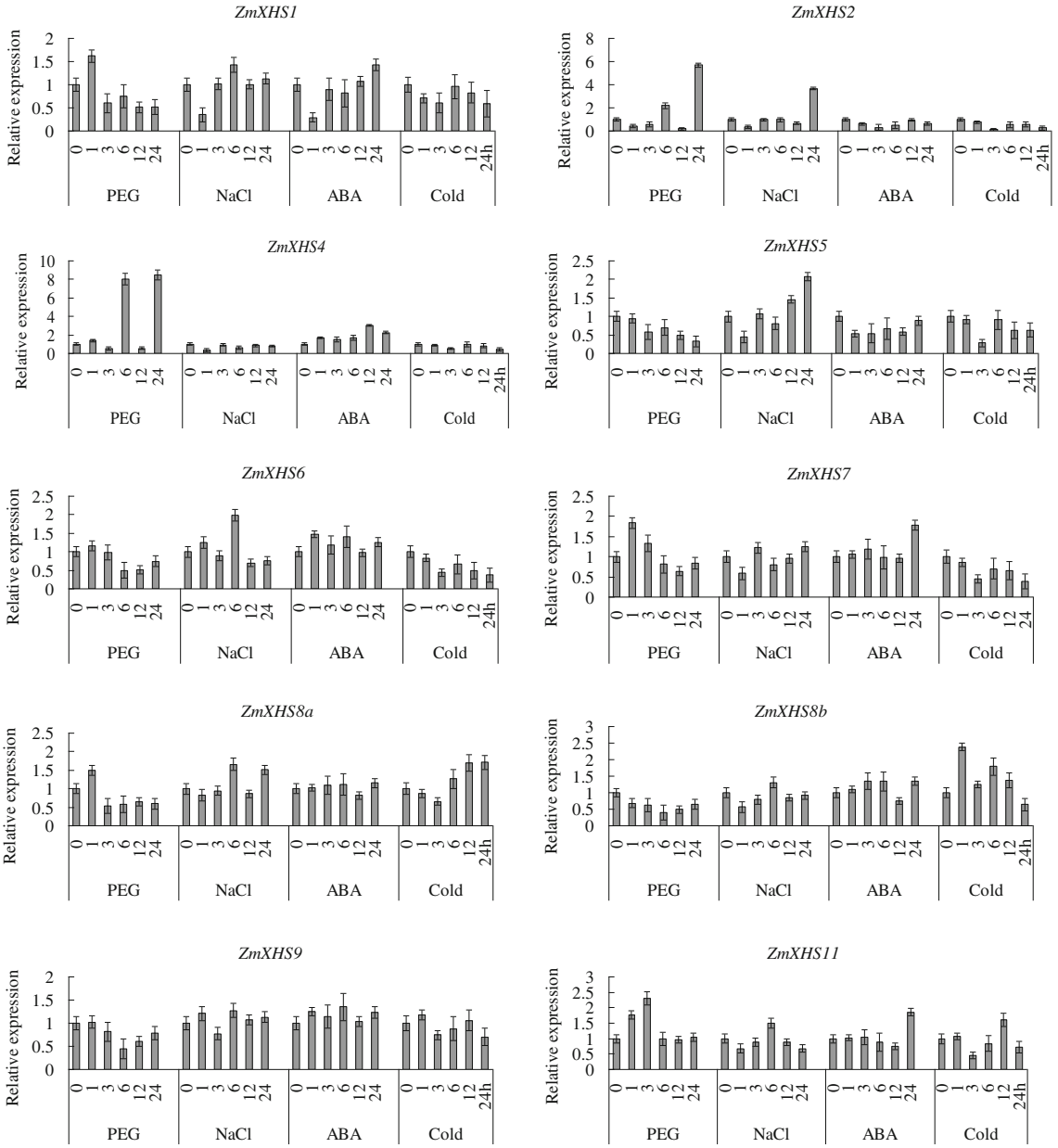

Fig. 6 Expression levels of maize *XHS* genes under each treatment, including ABA, cold, NaCl and PEG stress, based on real-time quantitative PCR. The *X*-axes are treatment time points and the *Y*-axes are scales of relative expression level. The transcript level at time 0 h (untreated) was used as the calibrator and was given as 1

genes were responsive to stresses in seedling stage, quantitative real-time PCR (qRT-PCR) was conducted to analyze all ten genes' transcriptional expression in shoots at the three-leaf stage treated by exogenous ABA, PEG, NaCl, and low temperature (4 °C).

The results indicated that, except for *ZmXHS*9, the other nine genes were induced in the seedling leaves by at least one of the four stresses applied (Fig. 6). Among them, six genes (*ZmXHS*1, *ZmXHS*2, *ZmXHS*4, *ZmXHS*7, and *ZmXHS*11) were obviously induced by PEG stress with distinct patterns. For example, the transcript levels of *ZmXHS*1, *ZmXHS*7, and *ZmXHS*11 were increased at the early stage of PEG stress and then decreased, while the expression level of *ZmXHS*2 and *ZmXHS*4 increased gradually and reached the highest level at the late stage of the stress. *ZmXHS*2, *ZmXHS*5, and *ZmXHS*6 were induced by salt stress, while *ZmXHS*1 and *ZmXHS*8b were suppressed by salt stress at 1 h after treatment. Under ABA stress condition, the expression level of *ZmXHS*4 increased gradually and peaked at 12 h, while that of the other nine gsenes were no obviously initiated. There was only one gene, *ZmXHS8b* responsive to low temperature (4 °C) treatment. The expression of *ZmXHS8b* was evidently induced within 1 h after low temperature treatment.

Expression profiles of *ZmXHS* genes under biotic stresses

To discover the *ZmXHS* (only seven genes were detected) genes involved in biotic stresses, we detected differentially expressed genes with microarray data. Data sets of three experiments under the pathogen treatments of *Fusarium moniliforme*, *Sphacelotheca reiliana*, and *Colletotrichum graminicola* infection have been analyzed. As shown in Fig. 7, *ZmXHS2* and *ZmXHS11* were downregulated after the infection of the three pathogens. *ZmXHS5* and ZmXHS8b were suppressed by *C. graminicola* and *S. reiliana*, respectively. *ZmXHS7* was suppressed by *F. moniliforme* and *C. graminicola* while induced by *S. reiliana* treatment. *ZmXHS1* was upregulated while *ZmXHS9* was downregulated by *C. graminicola* (Fig. 7).

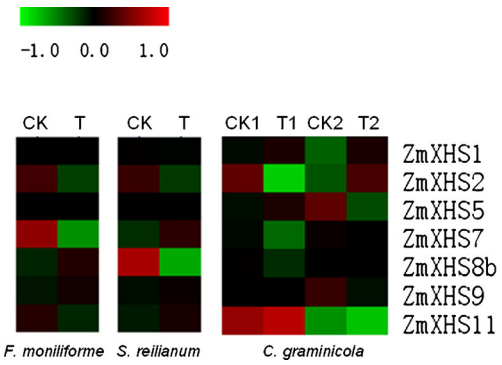

Fig. 7 Expression levels of maize *XHS* genes under biotic stresses. CK1: Samples from uninfected control plants were taken at the same time points of T1. *T1* Samples from infected leaves were taken at 36 h post infection. *CK2* Samples from uninfected control plants were taken at the same time points of T2. *T2* Samples from infected leaves were taken at 96 h post infection

Discussion

X1-homologous genes (*XHS*) encode plant specific proteins containing three basic domains (XH, XS, zf-XS) (Qin et al. 2009). In this study, ten genes belonging to the *XHS* in maize were identified. Among the better analyzed plant *XHS* families, *Arabidopsis* has likely 14 *XHS*, rice has at least 11 expressed *XHS* (Qin et al. 2009), sorghum at least 19 *XHS* (http://www.gramene.org/Multi/blastview). The genome structure of the *ZmXHS* genes showed that most them had six exons, except for *ZmXHS8a, 8b, 9* which had three (Fig. 2). Most rice and sorghum *XHS genes* also had six exons, but most *Arabidopsis XHS genes* had seven or eight exons (Supplementary Fig. 4). Five of ten proteins, ZmXHS1, ZmXHS2, ZmXHS4, ZmXHS5 and ZmXHS6 contained the XH domain which was necessary for proteins interaction. In *Arabidopsis*, the XH domain of FDM1 was necessary for FDM1–FDM1 and FDM1–IDN2 interactions (Xie et al. 2012a). In our study, the XH domain of ZmXHS5 was necessary for interacts with both itself and ZmXHS2 (Fig. 4).

Increasing evidence indicated that some gene families had vital roles both in development and response to stress (Qin et al. 2009). Examples were the genes from CBL-interacting protein kinase (CIPK) and F-BOX family and many transcription factor families such as NAC, MYB, and bZIP (Urao et al. 1993; Shi et al. 1999; Liu et al. 2000; Durfee et al. 2003; Hu et al. 2006; Kim 2006).

The members of XHS gene family in rice showed tissue-specific expression patterns. All the 11 *OXHS* genes were observably expressed in floral organs, and some were expressed in a wide range of different organs in rice (Qin et al. 2009). Like that in rice, the transcript levels of the ten *ZmXHS* genes were all had different expression in reproductive and vegetative organs (Fig. 5). The XS domain containing proteins are involved in a wide range of processes such as viral defense and stress response (Qin et al. 2009). In *Arabidopsis*, SGS3 was required for post-transcriptional gene silencing, natural virus resistance and *sgs3* mutant shown enhanced susceptibility to CMV (Mourrain et al. 2000). In tomato, SISGS3 was specifically required for the RNA-silencing defense against geminiviruses (Glick et al. 2008). In our work, most of *ZmXHS* were up or down regulated by the pathogen treatments of *F. moniliforme*, *S. reiliana*, and *C. graminicola.* In rice, nine *OXHS* genes were responsive to at least one of the abiotic stresses including drought, salt, cold, and abscisic acid treatment. Over-expression of the gene *OXHS2* in rice resulted in reduced tolerance to salt and drought stresses (Qin et al. 2009). In our study, except *ZmXHS*9, the other nine

genes were induced in the seedling leaves by at least one of the four stresses PEG cold, NaCl, or ABA (Fig. 6). In cluster III of phylogenetic tree (Supplementary Fig. 5), ZmXHS2 was the closest homologue to OXHS2, and the both proteins have strong similarity in domain composition, suggested that ZmXHS2 might be an orthologue of OXHS2 in maize. Both genes were induced by drought and salt treatment (Fig. 6) (Qin et al. 2009). These results indicated that ZmXHS2 might have the similar function to OXHS2.

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